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Sodium challenge does not support an acute gastrointestinal–renal natriuretic signaling axis in humans

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A gastrointestinal–renal natriuretic signaling axis has been proposed to regulate sodium excretion in response to acute sodium ingestion. Such an axis is thought to be regulated by a gastrointestinal sodium sensor coupled to the activation/release of a natriuretic signal and could have important clinical and scientific implications. Here we systematically tested for this putative axis and the potential involvement of the gastrointestinal-derived natriuretic prohormones prouroguanylin and proguanylin in 15 healthy volunteers. There was no difference in sodium excretion following equivalent oral or intravenous sodium loads during either high- or low-sodium diets. Furthermore, serum concentrations of prouroguanylin and proguanylin did not increase, did not differ following oral or intravenous sodium, and did not correlate with sodium excretion. Thus, our results do not support an acute gastrointestinal–renal natriuretic axis or a central role for prouroguanylin or proguanylin in humans. If such an axis does exist, it is not characterized by a significant difference in the pattern of sodium excretion following either an oral or intravenous sodium load.

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There has been renewed interest in a putative rapid-acting gastrointestinal (GI)–renal natriuretic signaling axis, which may have a key role in the acute regulation of renal sodium excretion in response to sodium ingestion.[1,2] The proposed mechanism of this natriuretic axis is that a dietary sodium load is detected by a GI sodium sensor that is coupled to the activation or release of a natriuretic signal, which in turn acts on the kidney to increase sodium excretion. Such an axis could have major clinical and scientific implications for sodium handling in health and disease.

Support for this GI–renal natriuretic axis arises primarily from the observation[3,4] that an equivalent sodium load is more rapidly excreted when given orally than when given intravenously. On the basis of this observation, it was suggested that the GI tract has the capacity to detect ingested sodium and subsequently release a natriuretic effector hormone into the circulation. Nevertheless, whether a GI–renal natriuretic axis exists in humans has not been firmly established. Furthermore, there is a lack of follow-up studies specifically conducted to confirm these important early observations. In addition, these early studies of oral versus intravenous (IV) sodium handling were conducted only during low sodium intake.

There has been recent intense interest in the small guanylyl cyclase–activating natriuretic peptide uroguanylin (UGN) and its inactive propeptide prouroguanylin (pro-UGN) as the leading candidate effector molecules for a putative GI–natriuretic axis linking dietary sodium intake to acute changes in sodium excretion.[1,2,5–31] The proposed mechanism for this axis is that the inactive prohormone pro-UGN is produced and stored in intestinal mucosa and is released into the circulation in response to sodium ingestion. Pro-UGN then circulates along with some preformed UGN[11,13] and is converted to its active form UGN in the kidney,[5,6] where it acts to directly promote sodium excretion. There is a similar mechanism that has been proposed for the prohormone

proguanylin (pro-GN) and its corresponding active hormone guanylin (GN), although pro-GN and GN have been less well studied.[8,9,17,26–28]

Nevertheless, although there is some experimental support that pro-UGN is secreted from intestinal mucosa under the influence of dietary sodium chloride, it has not yet been established whether serum pro-UGN concentration increases in response to a dietary sodium chloride load and whether the pro-UGN levels indeed correlate with sodium excretion in humans.

We sought to systematically investigate the existence in humans of the putative rapid-acting GI–renal natriuretic signaling axis and the potential role(s) of the GI-derived natriuretic prohormones pro-UGN and pro-GN as mediators of this axis. The specific objectives of this randomized experimental clinical trial conducted in healthy young human volunteers in a monitored inpatient clinical pharmacology research unit under both low and high sodium intakes were as follows: (1) to determine whether an oral sodium load is excreted more rapidly than sodium given intravenously during steady-state maintenance with either low- or high-sodium diets, thus supporting the existence of a GI–renal natriuretic axis; (2) to determine whether serum concentrations of pro-UGN and the less well-characterized proguanylin (pro-GN) increase acutely in response to a sodium load; (3) to determine whether the response in pro-UGN and pro-GN concentrations is greater following oral versus intravenous administration, thus supporting a GI-pro-UGN signaling axis; and (4) to determine whether changes in pro-UGN and pro-GN concentrations correlate with renal sodium excretion.

RESULTS

Baseline and demographic characteristics

Twenty-three potential subjects were screened. Eight did not qualify for the study. Two potential subjects had elevated liver enzymes at screening, one had elevated potassium and triglycerides, one had estimated Ccr <100 ml/min, and three indicated after screening that they would not be able to comply with the extended inpatient stays and dietary restrictions (Table 1).

Fifteen participants were enrolled and completed the first inpatient period and both sodium handling studies (Table 1). Eleven completed both phases including all four sodium handling studies. Of the 15 enrolled, 9 were men and 6 were women. The mean (s.d.) age of the group was 26.5 (2.9) years. The mean (s.d.) body mass index was 25.9 (4.1), the Cockroft–Gault-estimated glomerular filtration rate was 131.7 (32.1) ml/min, and the modification of diet in renal disease–estimated glomerular filtration rate was 116.5 (17.5) ml/min. The mean (s.d.) sodium excretion after 5 days of low sodium intake was 21 (9) mmol per 24 h and during the high-sodium period was 151 (43) mmol per 24 h. We observed a trend to an inverse correlation of basal urinary sodium excretion (UnaV) with aldosterone concentration but no correlation with either pro-UGN or pro-GN.

Sodium excretion in response to an IV versus oral sodium load

Cumulative and hourly sodium excretion for the entire 24-h acute sodium handling period for both the low- and high-sodium periods are shown in Figure 1a and b. Figure 1c and d display the first 6 h of Figure 1a and b, respectively. Cumulative sodium excretion for the entire 24-h period did not differ between the IV and oral administration studies for either the low sodium or the high-sodium periods. Similarly, neither hourly sodium excretion nor cumulative sodium excretion was significantly higher following oral administration at interim points of either the low-sodium or the high-sodium periods.

Individual sodium excretion curves

The individual sodium excretion curves for the 15 subjects who completed period 1 and the 11 subjects who completed both periods are shown in Figure 2. In reviewing the individual subject data, there appeared to exist little evidence for subjects to manifest a higher natriuretic response to oral versus IV administration.

Table 1 | Baseline and demographic characteristics

	Age (years)	Gender (M/F)	Race	BMI (kg/m^2)	SBP (mm Hg)	DBP (mm Hg)	C–G eGFR (ml/min)	MDRD eGFR (ml/min)
1	21	M	C	28.4	116	68	203	150
2	29	M	C	22.5	126	72	105	98
3	29	F	C	21.7	104	73	107	103
4	22	M	C	31.2	122	76	160	115
5	23	F	B	31.9	119	82	165	129
6	26	M	C	24.0	120	62	104	90
7	29	M	B	31.5	120	84	147	124
8	24	M	C	22.9	122	74	119	89
9	30	M	C	31.9	124	72	180	121
10	27	F	C	21.8	108	70	106	138
11	30	M	C	28.7	126	76	131	121
12	28	F	C	23.0	113	79	112	110
13	25	F	C	23.9	106	73	107	111
14	28	M	C	21.6	110	68	101	115
15	27	F	C	23.0	93	67	128	135

Abbreviations: B, Black; BMI, body mass index; C, Caucasian; C–G eGFR, Cockroft–Gault-estimated glomerular filtration rate; DBP, diastolic blood pressure; F, Female; M, male; MDRD eGFR, Modification of Diet in Renal Disease equation estimated glomerular filtration rate; SBP, systolic blood pressure.

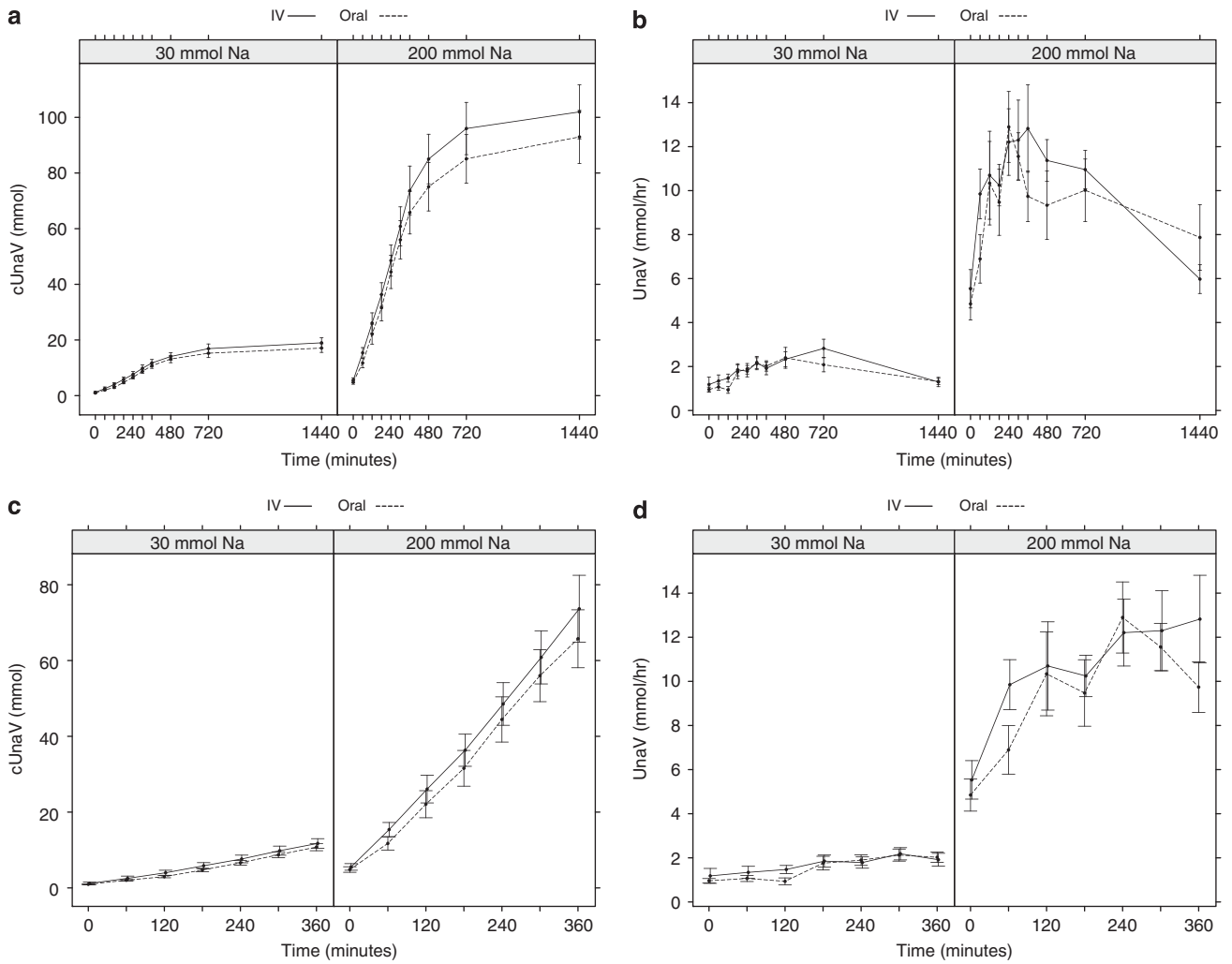

Figure 1 | Sodium excretion in response to an IV versus oral sodium load (Mean (s.e.m.)). IV, intravenous; cUnaV, cumulative urine sodium excretion; UnaV, urinary sodium excretion.

Aldosterone concentration response to oral versus IV sodium

Aldosterone concentration (ng/dl) response to oral versus IV sodium administration during the 6-h acute sodium handling study for both the high- and low-sodium periods is shown in Figure 3. Aldosterone concentration did not differ between oral and IV administration for either study period.

Serum pro-UGN and pro-GN concentrations in response to acute IV versus oral sodium load

The acute responses of pro-UGN and pro-GN concentrations to sodium administration during the 6-h acute sodium handling study for both the high-and low-sodium periods are shown in Figure 4a and b. During the low-sodium diet condition, serum pro-UGN concentration at 60 min post sodium administration had declined to 2.4 ng/ml with IV and 2.2 ng/ml with oral administration ($P<0.01$) and was not significantly different from baseline value by the midpoint of hour 6. During the high-sodium period, serum pro-UGN concentration at 60 min post sodium administration had declined to 2.1 with IV and 2.2 with oral administration ($P<0.001$) and remained depressed throughout the entire 6-h period. Pro-GN concentration did not change significantly in response to either IV or oral sodium administration for either the low- or high-sodium intake periods.

Correlation of fall in pro-UGN with sodium excretion

As secondary analysis, we investigated the relationship between pro-UGN concentration and sodium excretion via the Spearman correlation between the drop in pro-UGN over the first 90 min and cumulative sodium excretion for the 6-h acute sodium handling study (see Figure 5), and did not observe evidence of a relationship (IV/high diet: $r=0.1$. ($P=0.78$), oral/high diet: $r=0.21$ ($P=0.54$), IV/low diet: $r=0.07$ ($P=0.79$), oral/low diet: $r=0.21$ ($P=0.44$)). In addition, we further investigated the joint evolution of pro-UGN concentration and UnaV for oral administration (high diet) via the correlation of pro-UGN and UnaV slope random effects within a multivariate mixed-effects

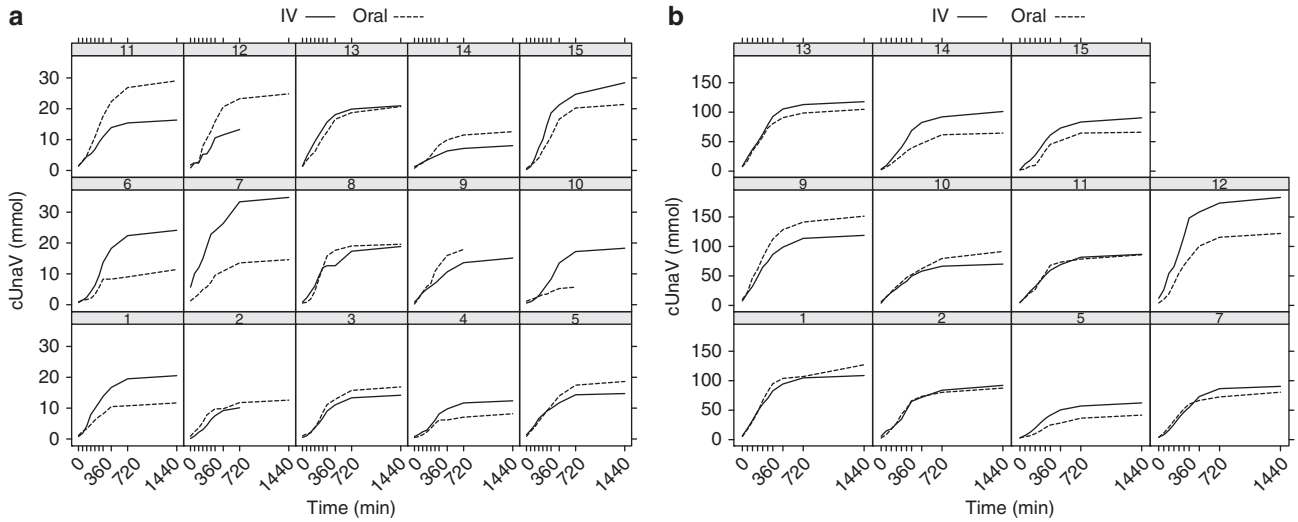

Figure 2 | Individual sodium excretion curves. (**a**) 30 mmol Na diet. (**b**) 200 mmol Na diet. IV, intravenous; cUnaV, cumulative sodium excretion rate.

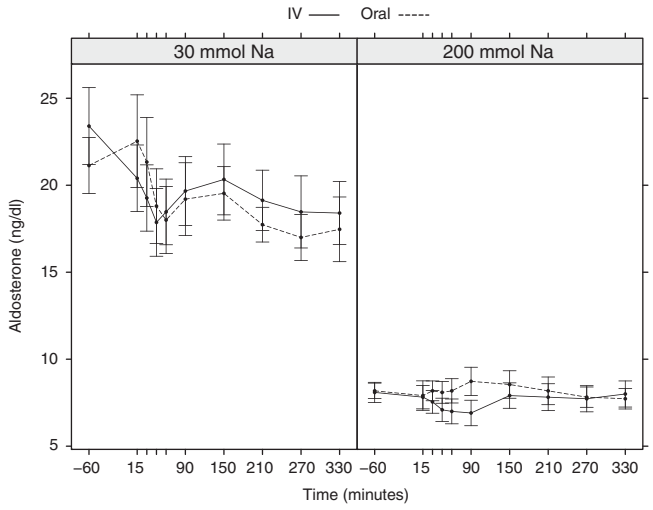

Figure 3 | Aldosterone response to oral versus intravenous (IV) sodium.

model.[32,33] This correlation was −0.73 and the likelihood ratio test for correlated random effects between pro-UGN concentration and UnaV yielded $P = 0.18$, thereby not providing strong evidence for the joint evolution hypothesis. In summary, we did not find strong evidence for a correlation between the decrease in pro-UGN concentration and the increase in sodium excretion.

Blood pressure and creatinine clearance

Systolic and diastolic blood pressure versus time during the sodium handling study is shown in Figure 6. Blood pressure did not change significantly following the saline challenge. Creatinine clearance (Ccr, ml/min) is shown in Figure 7. On the 200 mmol/day diet, Ccr increased following the saline challenge but did not differ between oral and IV sodium administration. The increase in Ccr occurred at roughly the same time point as the drop in pro-UGN concentration. Thus, an increase in glomerular filtration rate could have possibly resulted in increased filtration of pro-UGN, although this drop was not observed for pro-GN.

DISCUSSION

We found no difference in the cumulative or hourly natriuretic response to oral versus IV sodium administration during either a standard 30 mmol Na diet or a standard 200 mmol Na diet in healthy human volunteers. Therefore, our results do not confirm the earlier findings of a more rapid sodium excretion following oral versus IV administration and do not support the existence of an acute GI–renal natriuretic signaling axis. Our study was comparable to an earlier study,[4] which maintained subjects on the standardized diet for 4 days before the sodium handling study that was conducted on day 5. In our study, subjects were housed in our clinical pharmacology research unit on the controlled diet for 5 days before the first Na handling study, which was conducted on day 6. Our subjects then continued the controlled diet for 3 more days as a recovery period before the second sodium handling study, which was conducted on day 10.

It can be argued that the finding of equal excretion following oral versus IV sodium administration does not entirely exclude the existence of such an axis. The IV administration would theoretically result in a nearly immediate expansion of the extracellular fluid volume and stimulation of rapid-acting natriuretic mechanisms. Oral administration, however, would require absorption across the GI mucosa, and travel across the GI vascular bed to the central veins before expanding the extracellular volume. Such a delay could theoretically manifest as a slower excretion. The finding of equal rates of excretion may therefore suggest that such an axis may exist. Nevertheless, we were unable to confirm the existence of this axis using our rigorous sodium challenge protocol.

Our results do not substantiate the long-held notion that an oral saline load is significantly more rapidly excreted than

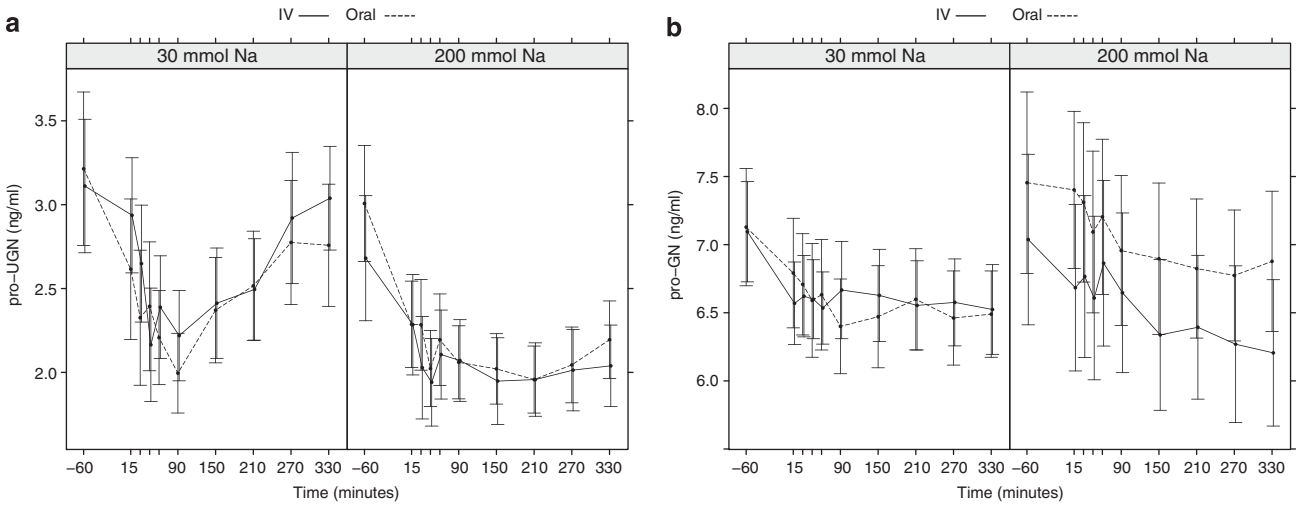

Figure 4 | Serum prouroguanylin and proguanylin levels in response to acute intravenous (IV) versus oral sodium load. pro-GN, prohormone proguanylin; pro-UGN, propeptide prouroguanylin.

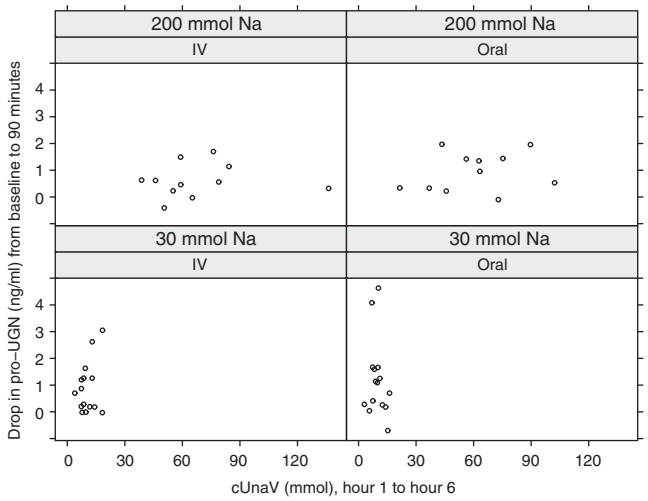

Figure 5 | Correlation of fall in prohormone proguanylin (pro-UGN) with Na excretion (high sodium period). cUnaV, cumulative sodium excretion rate; IV, intravenous; pro-UGN, propeptide prouroguanylin.

the equivalent IV load. The earlier studies found that salt-depleted individuals consuming a 10-mmol sodium diet who were given 100 mmol oral sodium chloride had higher cumulative sodium excretion over 24 h than individuals given the same amount of sodium intravenously. However, differences between oral and IV administration were small after 2 h and became increasingly more pronounced with each time interval over the ensuing 24-h period. The difference between cumulative UnaV paradoxically becoming greater after 8–12 h does not seem consistent with an acute, rapid-acting signaling mechanism in which the difference would be most pronounced immediately or shortly after the sodium load.

We performed the oral versus IV sodium handling studies during the same admission period under exactly the same dietary conditions. The earlier study separated the sodium handling experiments by an outpatient period and was conducted only during low (10 mmol per 24 h) sodium intake. Conducting sodium handling studies only during a low basal sodium intake is problematic given that under these conditions natriuretic mechanisms would be blunted. Our sodium handling studies were conducted under both low (30 mmol) and high (200 mmol) sodium intakes that were selected to represent the spectrum of physiological sodium intakes. We selected 30 mmol rather than 10 mmol because it represents the extreme lower limit of sodium intake in a reasonable physiological range. The average urinary excretion of sodium after 5 days suggests an average intake of 21 (9) mmol per 24 h. Neither the high nor low dietary sodium confinement condition revealed a difference between oral and IV sodium administration. Consistent with the earlier experiments, however, serial aldosterone concentrations did not differ regardless of whether the sodium load was given orally or intravenously.

Although the early studies showed a marked difference between the rates of sodium excretion following oral versus IV sodium, and some follow-up studies support these findings,[32] other studies did not always find such a difference.[33] Therefore, the existence of this axis has been somewhat controversial.[1] We should also point out that our negative findings do not disprove the existence of a GI–renal sodium axis. The GI tract may indeed detect changes in sodium intake, but our investigation did not find that this sensor manifests as a difference in the natriuretic response to oral versus IV sodium. It should be mentioned that aldosterone concentration has a diurnal variation, generally highest in the morning and falling throughout the day and night.[34] There is little data, however, on the diurnal variation in pro-UGN and pro-GN,[29,35] although one study suggests that levels are highest in humans in the morning.[29] Basal aldosterone, pro-GN, and pro-UGN in our study

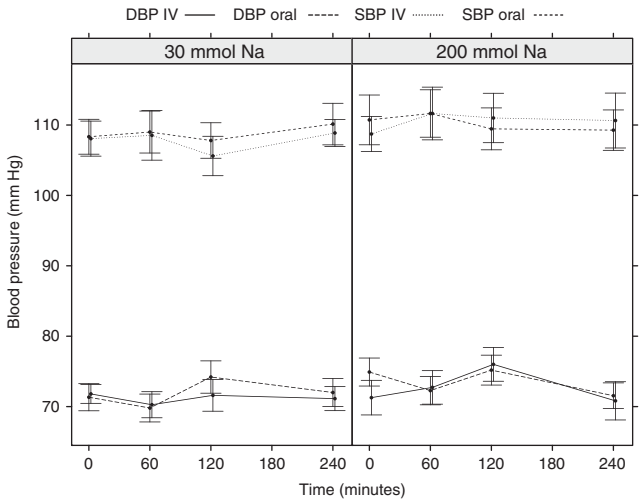

Figure 6 | Blood pressure (mm Hg). DBP, diastolic blood pressure; IV, intravenous; SBP, systolic blood pressure.

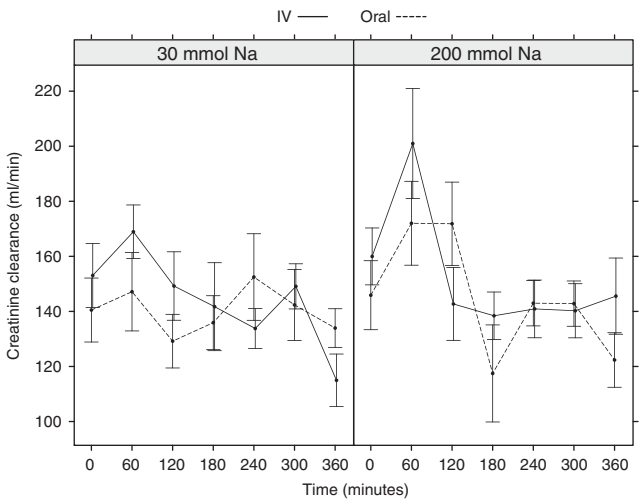

Figure 7 | Creatinine clearance (ml/min). IV, intravenous.

demonstrated inter-subject variation consistent with existing data (Figures 3 and 4a, b).

To our knowledge, this was the first study of changes in pro-UGN and pro-GN concentrations in response to sodium loading in humans. By comparing IV versus oral sodium, we sought to differentiate whether there was a GI sensor causing pro-UGN and pro-GN release. We did not find evidence of such a release mechanism. On the contrary, we found that pro-UGN concentrations decreased in response to both oral and IV sodium administration. This decrease could possibly reflect increased glomerular filtration of pro-UGN and intra-renal conversion to UGN. Nevertheless, the reduction in pro-UGN was observed equally in response to both IV and oral sodium. Our secondary statistical analysis did not find evidence for a correlation between the decrease in pro-UGN concentration and sodium excretion.

Although our data do not support the hypothesis that an ingested sodium load leads to the acute release of pro-UGN via a GI-sensing mechanism, there is a long line of experimental evidence supporting a substantial natriuretic effect of UGN.[20–25] Moreover, disruption of endogenous UGN in mice results in impaired excretion of oral sodium and hypertension.[25]

Nevertheless, in our randomized physiological study, we did not find a difference in sodium excretion or changes in pro-UGN or pro-GN concentrations in response to oral versus IV sodium administration. Furthermore, we did not observe an increase in pro-UGN or pro-GN concentrations in response to either oral or IV sodium. These observations provide evidence that the role of the pro-UGN/UGN system in sodium balance may be more complex than the existing leading hypotheses have indicated. In fact, we observed a decrease in pro-UGN (but not pro-GN) concentration in response to both oral and IV sodium.

Our randomized study, conducted in an inpatient clinical pharmacology unit under close dietary control, provides key data that help clarify scientifically and clinically important questions regarding sodium metabolism in humans. Our results suggest that if a GI–renal sodium axis does indeed exist, this axis does not manifest as a significant difference in the pattern of sodium excretion following a sodium load administered either orally or intravenously. Moreover, this axis does not appear to be mediated via increased release of pro-UGN or pro-GN.

MATERIALS AND METHODS

In this randomized physiological clinical trial, we determined stimulated hourly and cumulative renal sodium excretion following an oral sodium chloride challenge of 102.6 mmol versus an IV sodium chloride challenge of 102.6 mmol in healthy volunteers. In addition, we simultaneously determined the stimulated hourly pro-UGN and pro-GN serum concentrations. These determinations were made both during a standard low-sodium diet (30 mmol/day Na, 60 mmol/day K) and while receiving a standard high-sodium diet (200 mmol/day Na, 60 mmol/day K). The rationale for this design was that if a GI–renal natriuretic axis existed, sodium excretion would be greater with oral than with IV administration. In addition, if pro-UGN release is the mediator of this axis, we would anticipate greater release of pro-UGN with oral than IV sodium. We further hypothesized that these findings would be augmented on a high versus a low sodium intake.

Primary end points

(1) Hourly (UnaV, mmol/h) and cumulative sodium excretion rates (cUnaV, mmol) at baseline and following an oral versus an IV 102.6 mmol Na challenge during 30 mmol/day and 200 mmol/day sodium diets.
(2) Hourly pro-UGN and pro-GN serum concentrations at baseline and following an oral versus an IV 102.6 mmol Na challenge during 30 mmol/day and 200 mmol/day sodium diets.

Secondary end points

(1) Hourly sodium excretion rate (UnaV, mmol/h) following an oral versus an IV 102.6 mmol Na challenge during 30 mmol/day and 200 mmol/day sodium diets.

(2) Hourly aldosterone concentration (ng/dl) following an oral versus an IV 102.6 mmol Na challenge during 30 mmol/day and 200 mmol/day sodium diets.

Study participants

The study was conducted by the Principal Investigator and study team in accordance with the guidelines of the University of Miami Human Subjects Research Office (Institutional Review Board) and the Principles of the Declaration of Helsinki. Written informed consent was obtained by study coordinators directly from all participants before their entry into the study and before initiating any study procedures. Subjects were recruited from a large database of healthy volunteers and were eligible if they were between the ages of 20 and 30 years and had no significant medical history. Qualified participants had a normal physical examination, blood pressure $<130/80$, and normal laboratory evaluation including a Cockroft–Gault-estimated glomerular filtration rate >100 ml/min.

Study design and overview

This study consisted of a screening and eligibility visit followed by two sequential 10-day confinement periods in the Clinical Pharmacology Research Unit (CPRU) at the University of Miami. The confinement periods were separated by an outpatient period of at least 1 week, during which the study participants consumed their usual diet. During the first 10-day confinement period, the participants received a 30-mmol sodium 60-mmol K diet. During the second 10-day confinement period, the participants received a 200-mmol sodium 60-mmol K diet. After diet stabilization during confinement days 1–5, two 24-h sodium handling studies, one with IV sodium load and the other with oral sodium load, were conducted in random order on days 6 and 10. Meals were identical and consumed at exactly the same times on days 6 and 10 of each diet period during the 24-h sodium handling study. In addition, meals were identical on days 3 and 7; 4 and 8; and 5 and 9.

Sodium handling study procedure for days 6 and 10 of each diet period

Each participant fasted overnight from 2200 hours of the previous night except for *ad libitum* water intake. At approximately 0630 hours, two 500-ml water loads were followed directly by a 2-h baseline control urine collection. At the midpoint of the 2-h baseline urine collection, blood was taken to analyze pro-UGN, pro-GN, creatinine, sodium, potassium, and aldosterone concentrations. Urine volume for this 2-h period was recorded and samples analyzed for creatinine, sodium, and potassium.

Following the 2-h baseline control urine collection, participants received one of the following two experimental test procedures in random order:

(1) 102.6 mmol oral sodium as sodium chloride tablets administered over a 10–15-min period with 240 ml of sugar-free diet soft drink and an additional 200 ml water.
(2) 102.6 mmol IV sodium as 3% saline (513 mmol/l) administered over a 10–15-min period also with 240 ml of sugar-free diet soft drink.

Following the NaCl load administration, diuresis was sustained by hourly administration of 200 ml of water given at the beginning of each of 6-hourly urine collection periods. Urine samples for creatinine, sodium, and potassium were collected for six additional 1-h periods. These periods terminated at 60, 120, 180, 240, 300, and 360 min following the administration of the sodium load. Following the hour 6 urine collection, urine was collected and volume recorded at intervals 6–8 h, 8–12 h, and 12–24 h. Blood was taken at 15, 30, 45, 60, 90, 150, 210, 270, and 330 min following the sodium load for pro-UGN, pro-GN, creatinine, sodium, potassium, and aldosterone concentrations.

Safety monitoring

The study participants were monitored closely in the locked, secured, inpatient CPRU of the Division of Clinical Pharmacology for signs of volume overload. Furosemide 20 mg ampoules for IV administration were readily available on site in case of acute volume overload.

Analytical methods

Pro-UGN and pro-GN concentrations. Samples for pro-UGN and pro-GN were collected in serum separator tubes and centrifuged immediately in a refrigerated centrifuge. They were frozen and stored at $-20\,^{\circ}C$. Samples were analyzed within 1–2 weeks of being drawn. Concentrations of pro-UGN (ng/ml) and pro-GN (ng/ml) were determined in the serum samples using the Human Prouroguanylin enzyme-linked immunosorbent assay (ELISA) Immunoassay (RD191069200R, Biovendor, Modrice, Czech Republic) and the Human Proguanylin ELISA Immunoassay (RD191046100R, Biovendor) according to their assay directions. In brief, pro-UGN or pro-GN ELISA standards, quality controls, and serum samples are incubated in the wells of microtitration plates that have been coated with anti-pro-UGN or anti-pro-GN antibodies. After washing the wells thoroughly, biotin-labeled polyclonal anti-pro-UGN or anti-pro-GN antibodies are added to the wells and incubated for 1 h. The wells are washed and then a solution containing a conjugate of streptavidin–horseradish peroxidase is added. Following 30 min of incubation, the wells are washed and then the substrate, H_2O_2–tetramethybenzidine, is added and the resulting product is measured 450 nm in a plate reader. Standard curves for authentic pro-UGN and pro-GN are constructed according to the manufacturer's instructions.

Aldosterone concentration

Aldosterone concentration (ng/dl) was determined by coated-tube radioimmunoassay.

Statistical methods

A sample size of 15 study participants allows the detection of a 1 s.d. difference (oral versus IV; estimated 1.8 mmol) in mean hour 4 and hour 6 cUnaV with a power of 0.90 and family-wise alpha 0.05 (each end point separately powered at alpha $= 0.025$). A power of 0.80 is achieved under these assumptions with a sample size of 11 participants. Considering the possibility for subject withdrawals, a conservative enrollment was considered to be 15 subjects. A sample size of eight subjects was used in a previous study of Na handling,[3,4] yielding a large difference between oral and IV sodium administration.

Summary statistics were calculated for demographic, clinical, blood pressure, and biochemical measures. Differences in renal sodium handling between the two interventions on both high and low sodium intakes were detected using a paired *t*-test, along with bootstrap confidence intervals. As secondary analysis, we investigated the relationship between pro-UGN and sodium excretion in response to sodium administration via the Spearman correlation

between the drop in pro-UGN over the first 90 min and the cumulative sodium excretion for the 6-h acute sodium handling study. Multivariate mixed-effects models were also used to assess the joint evolution of pro-UGN and pro-GN via correlation of slope random effects.[36–38] All statistical analyses were performed with R statistical software, and the nlme R package was used to estimate multivariate mixed-effects models via restricted maximum likelihood.[39,40]

DISCLOSURE

All the authors declared no competing interests.

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REFERENCES

1. Michell AR, Debnam ES, Unwin RJ. Regulation of renal function by the gastrointestinal tract: potential role of gut-derived peptides and hormones. *Annu Rev Physiol* 2008; **70**: 379–403.
2. Thomas L, Kumar R. Control of renal solute excretion by enteric signals and mediators. *J Am Soc Nephrol* 2008; **19**: 207–212.
3. Carey RM. Evidence for a splanchnic sodium input monitor regulating renal sodium excretion in man: lack of dependence upon aldosterone. *Circ Res* 1978; **43**: 19–23.
4. Lennane RJ, Carey RM, Goodwin TJ *et al.* A comparison of natriuresis after oral and intravenous sodium loading in sodium-depleted man: evidence for a gastrointestinal or portal monitor of sodium intake. *Clin Sci Mol Med* 1975; **49**: 437–440.
5. Moss NG, Fellner RC, Qian X *et al.* Uroguanylin, an intestinal natriuretic peptide, is delivered to the kidney as an unprocessed propeptide. *Endocrinology* 2008; **149**: 4486–4498.
6. Qian X, Moss NG, Fellner RC *et al.* Circulating prouroguanylin is processed to its active natriuretic form exclusively within the renal tubules. *Endocrinology* 2008; **149**: 4499–4509.
7. Forte LR. A novel role for uroguanylin in the regulation of sodium balance. *J Clin Invest* 2003; **112**: 1138–1141.
8. Forte Jr LR. Uroguanylin and guanylin peptides: pharmacology and experimental therapeutics. *Pharmacol Ther* 2004; **104**: 137–162.
9. Forte LR, London RM, Freeman RH *et al.* Guanylin peptides: renal actions mediated by cyclic GMP. *Am J Physiol Renal Physiol* 2000; **278**: F180–F191.
10. Hamra FK, Forte LR, Eber SL *et al.* Uroguanylin: structure and activity of a second endogenous peptide that stimulates intestinal guanylate cyclase. *Proc Natl Acad Sci USA* 1993; **90**: 10464–10468.
11. Hess R, Kuhn M, Schulz-Knappe P *et al.* GCAP-II: isolation and characterization of the circulating form of human uroguanylin. *FEBS Lett* 1995; **374**: 34–38.
12. Li Z, Perkins AG, Peters MF *et al.* Purification, cDNA sequence, and tissue distribution of rat uroguanylin. *Regul Pept* 1997; **68**: 45–56.
13. Fan X, Hamra FK, Freeman RH *et al.* Uroguanylin: cloning of preprouroguanylin cDNA, mRNA expression in the intestine and heart and isolation of uroguanylin and urouroguanylin from plasma. *Biochem Biophys Res Commun* 1996; **219**: 457–462.
14. Miyazato M, Nakazato M, Matsukura S *et al.* Uroguanylin gene expression in the alimentary tract and extra-gastrointestinal tissues. *FEBS Lett* 1996; **398**: 170–174.
15. Perkins A, Goy MF, Li Z. Uroguanylin is expressed by enterochromaffin cells in the rat gastrointestinal tract. *Gastroenterology* 1997; **113**: 1007–1014.
16. Nakazato M, Yamaguchi H, Date Y *et al.* Tissue distribution, cellular source, and structural analysis of rat immunoreactive uroguanylin. *Endocrinology* 1998; **139**: 5247–5254.
17. Carrithers SL, Jackson BA, Cai WY *et al.* Site-specific effects of dietary salt intake on guanylin and uroguanylin mRNA expression in rat intestine. *Regul Pept* 2002; **107**: 87–95.
18. Kinoshita H, Fujimoto S, Nakazato M *et al.* Urine and plasma levels of uroguanylin and its molecular forms in renal diseases. *Kidney Int* 1997; **52**: 1028–1034.
19. Fukae H, Kinoshita H, Fujimoto S *et al.* Changes in urinary levels and renal expression of uroguanylin on low or high salt diets in rats. *Nephron* 2002; **92**: 373–378.
20. Forte LR, Fan X, Hamra FK. Salt and water homeostasis: uroguanylin is a circulating peptide hormone with natriuretic activity. *Am J Kidney Dis* 1996; **28**: 296–304.
21. Greenberg RN, Hill M, Crytzer J *et al.* Comparison of effects of uroguanylin, guanylin, and Escherichia coli heatstable enterotoxin STa in mouse intestine and kidney: evidence that uroguanylin is an intestinal natriuretic hormone. *J Investig Med* 1997; **45**: 276–282.
22. Carrithers SL, Hill MJ, Johnson BR *et al.* Renal effects of uroguanylin and guanylin *in vivo*. *Braz J Med Biol Res* 1999; **32**: 1337–1344.
23. Fonteles MC, Greenberg RN, Monteiro HS *et al.* Natriuretic and kaliuretic activities of guanylin and uroguanylin in the isolated perfused rat kidney. *Am J Physiol* 1998; **275**(Pt 2): F191–F197.
24. Carrithers SL, Ott CE, Hill MJ *et al.* Guanylin and uroguanylin induce natriuresis in mice lacking guanylyl cyclase-C receptor. *Kidney Int* 2004; **65**: 40–53.
25. Lorenz JN, Nieman M, Sabo J *et al.* Uroguanylin knockout mice have increased blood pressure and impaired natriuretic response to enteral NaCl load. *J Clin Invest* 2003; **112**: 1244–1254.
26. Sindic A, Schlatter E. Cellular effects of guanylin and uroguanylin. *J Am Soc Nephrol* 2006; **17**: 607–616.
27. Sindic A, Velic A, Basoglu C *et al.* Uroguanylin and guanylin regulate transport of mouse cortical collecting duct independent of guanylate cyclase C. *Kidney Int* 2005; **68**: 1008–1017.
28. Sindic A, Hirsch JR, Velic A *et al.* Guanylin and uroguanylin regulate electrolyte transport in isolated human cortical collecting ducts. *Kidney Int* 2005; **67**: 1420–1427.
29. Carrithers SL, Eber SL, Forte LR *et al.* Increased urinary excretion of uroguanylin in patients with congestive heart failure. *Am J Physiol* 2000; **278**: H538–H547.
30. Sindic A, Schlatter E. Renal electrolyte effects of guanylin and uroguanylin. *Curr Opin Nephrol Hypertens* 2007; **16**: 10–15.
31. Sindic A, Schlatter E. Mechanisms of action of uroguanylin and guanylin and their role in salt handling. *Nephrol Dial Transplant* 2006; **21**: 3007–3012.
32. Singer DRJ, Markandu ND, Buckley MG *et al.* Contrasting endocrine responses to acute oral compared with intravenous sodium loading in normal humans. *Am J Physiol (Renal Physiol)* 1998; **274**: F111–F119.
33. Gordon D, Peart WS. Sodium excretion in man, and adaptations to a low-sodium diet: effect of intravenous sodium intake. *Clin Sci* 1979; **57**: 225–231.
34. Hurwitz S, Cohen RJ, Williams GH. Diurnal variation of aldosterone and plasma renin activity: timing relation to melatonin and cortisol and consistency after prolonged bed rest. *J Appl Physiol* 2004; **96**: 1406–1414.
35. Scheving LA, Jin WH. Circadian regulation of uroguanylin and guanylin in the rat intestine. *Am J Physiol* 1999; **277**(Pt 1): C1177–C1183.
36. Morrell C, Brant L. Using multivariate mixed-effects models to predict hypertension. *Proc Joint Stat Meeting Biometrics Sec* 2003: 2916–2921.
37. Fieuws S, Verbeke G. Joint modeling of multivariate longitudinal profiles: pitfalls of the random-effects approach. *Stat Med* 2004; **23**: 3093–3104.
38. Heitjan D, Sharma D. Modelling repeated-series longitudinal data. *Stat Med* 1997; **16**: 347–355.
39. R Project 2009. R—Version 2.9.1. Vienna: R Project (available from http://www.r-project.org).
40. Pinheiro J, Bates D. *Mixed-Effects Models in S and S-Plus*. Springer: New York, 2004.